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REVIEW

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Intestinal microbiota and colorectal cancer: changes in the intestinal microenvironment and their relation to the disease

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ABSTRACT

Tools that predict the risk of colorectal cancer are important for early diagnosis, given the high mortality rate for this cancer. The composition of the intestinal microbiota is now considered to be a risk factor for the development of colorectal cancer. This discovery has motivated a growing number of studies to identify the micro-organisms responsible for the onset and/or progression of colorectal cancer. With this in mind, this review discusses the relationship between the composition of the intestinal microbiota and colorectal cancer risk. Prospective and case–control studies indicate that the intestinal microbiota of individuals with colorectal cancer usually contains a greater proportion of bacteria responsible for gastrointestinal tract inflammatory diseases, as well as bacteria that produce toxins and carcinogenic metabolites. In contrast, there tends to be a reduced presence of butyric acid-producing bacteria, probiotic bacteria and potentially probiotic bacteria. Despite these differences, the onset and development of colorectal cancer cannot be attributed to a specific micro-organism. Thus, studies focused on the formation of the intestinal microbiota and factors that modulate its composition are important for the development of approaches for colorectal cancer prevention.

INTRODUCTION

Colorectal cancer (CRC) is the result of the accumulation of genetic and/or epigenetic alterations that lead colonocytes to show uncontrolled hyperplasia and dysplasia [1]. It is the third leading cause of cancer deaths worldwide, and is characterized as a global public health problem [2].

The recent literature highlights the composition of the intestinal microbiota as one of the risk factors for CRC. This risk was first observed by Reddy in 1974 when experimental animals kept under germ-free conditions did not develop colonic tumours induced by 1,2-dimethylhydrazine (10 mg/week/kg of body weight, for 20 weeks), but 17% of the animals kept under conventional conditions developed chemically induced adenocarcinomas [3]. Since then, similar results found in other experimental studies [4–6] have reinforced the hypothesis that some micro-organisms can increase the risk of developing CRC.

In 2011, Sears and Pardoll proposed that certain micro-organisms of the intestinal microbiota with unique virulence characteristics, known as alpha-bugs, could be pro-oncogenic. According to the authors, these micro-organisms can modulate the composition of the intestinal microbiome by reducing beneficial microbial groups. Thus, alpha-bugs can activate a chronic immune response that could provoke genetic and/or epigenetic changes in the colonocytes, culminating in the development of CRC [7].

Tjalsma *et al.* [8] proposed a bacterial model based on the alpha-bugs hypothesis. The driver–passenger model associates different types of bacteria with the pathogenesis of CRC along its temporal development. Initially, some types of bacteria, known as drivers (analogous to alpha-bugs), would damage colonocyte DNA and initiate carcinogenesis. Subsequently, because of tumour development and the consequent changes in the intestinal microenvironment, opportunist

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Keywords: *Fusobacterium*; bacterial metabolites; toxins; inflammation.
Abbreviations: *APC*, *adenomatous polyposis coli*; BFT, *Bacteroides fragilis* toxin; BMI, body mass index; CEACAM6, carcinoembryonic antigen-related cell adhesion molecule 6; COX-2, cyclooxygenase 2; CRC, colorectal câncer; DGGE, denaturing gradient gel electrophoresis; DNA-PCR; RISA, ribosomal intergetic spacer analysis; DSBs, DNA double-strand breaks; ERIC-PCR, enterobacterial repetitive intergenic consensus sequence-PCR using primer; ETBF, enterotoxigenic *Bacteroides fragilis*; FANCD2, fanconi anaemia protein D2; FISH, clone sequence analysis and florescence in situ hybridization; H_2O_2, hydrogen peroxide; ICLs, interstrand cross-links; IL, interleukin; LAB, lactic acid bacteria; MMP, matrix metallo proteinase; MMR, DNA mismatch repair; NF-κB, nuclear factor kappa B; NTBF, non-toxin-producing *Bacteroides fragilis*; PAN, proliferation of cell nuclear antigen; pks, polyketide synthase islands; qPCR, quantitative polymerase chain reaction; RAPD-PCR, random amplified polymorphic; ROS, reactive oxygen species; SCFAs, short -chain fatty acids; Sgg, *Streptococcus gallolyticus* subsp. *gallolyticus*; TNF-α, tumour necrosis factor alpha; T-RFLP, terminal restriction fragment length polymorphism.

Table 1. Main results of prospective studies that evaluated the intestinal microbiota of individuals with CRC

Subjects	Sample	Methodology	Main results		References
			Greater abundance	Lower abundance	
20 Irish patients with proximal carcinoma	Tumour tissue	qPCR of the 16S rRNA amplicons covering variable regions V3 to V4	Genera *Blautia*, *Clostridium* and *Faecalibacterium*	–	[9]
39 Irish patients with distal and rectal carcinoma	Tumour tissue	qPCR of the 16S rRNA amplicons covering variable regions V3 to V4	Genera *Alistipes*, *Akkermansia*, *Halomonas* and *Shewanella*	–	[9]
15 Chinese patients with proximal carcinoma that have not been submitted to chemotherapy or radiation treatments	Tumour tissue	Pyrosequencing of the 16S rRNA amplicons covering variable regions V3	Genera *Peptostreptoccus*, *Prevotella, Pyramidobacterium* and *Selenomonas*	–	[10]
16 Chinese patients with distal carcinoma that have not been submitted to chemotherapy or radiation treatments	Tumour tissue	Pyrosequencing of the 16S rRNA amplicons covering variable regions V3	Genera *Escherichia–Shigella, Fusobacterium* and *Leptotrichia*	–	[10]
1069 Americans with carcinoma	Tumour tissue	qPCR	Species *Fusobacterium nucleatum*	–	[11]

qPCR, quantitative polymerase chain reaction

bacteria, known as passengers, would proliferate and colonize this region. These kinds of bacteria contribute either to the progression or inhibition of tumour development. Thus, there is continuous replacement of driver bacteria by passenger bacteria over time [8].

Based on this phenomenon, a growing number of studies have focused their efforts on identifying micro-organisms in the intestinal microbiota and assessing their association with the risk of developing CRC (Tables 1–4), since early detection of these micro-organisms could help monitor CRC risk. In this context, the present review aims to discuss the relationship between the composition of the intestinal microbiota and the risk of developing CRC, as well as its associated mechanisms.

Bacteria involved in inflammatory diseases of the gastrointestinal tract

Chronic inflammation can predispose an individual to carcinogenesis. In general, any event that initiates or maintains intestinal inflammation can be considered to be a potential promoter of carcinogenesis [45]. Therefore, pro-inflammatory response triggered by interactions between the intestinal microbiota, the immune system and colonocytes may contribute to the development of CRC [46].

According to Sears and Pardoll [7], alpha-bugs trigger a chronic intestinal inflammation through a series of different mechanisms that can cause genetic alterations in colonocytes, and bacteria related to the development of inflammatory diseases of the gastrointestinal tract (periodontal disease, inflammatory bowel disease and appendicitis) have been found in larger amounts in individuals with CRC (Tables 1–4). An example is *Fusobacterium*, an aerobic Gram-negative bacterium, typically present in the oral cavity and rarely found in the intestinal microbiota of healthy individuals, and associated with periodontal diseases [47].

It is estimated that individuals with high amounts of *Fusobacterium* in their intestinal microbiota are 3.5 times more likely to develop colonic adenomas [19]. An increased population of *Fusobacterium* in the adenomas could increase the risk of developing colonic carcinomas, even for subtle increases in population [16]. Furthermore, the presence of *Fusobacterium nucleatum* in tumour tissue has been associated with lymph node metastases, a worse prognosis of the disease with a very low survival rate [11, 16, 40].

Fluorescence *in situ* hybridization has shown that *Fusobacterium* can be found beneath the colonic mucus layer, in direct contact with the colonocytes. Due to its location, *Fusobacterium* would be able to invade the colonic submucosa and induce a local inflammation [19, 48]. The adhesion and invasion of the intestinal submucosa occurs through the binding of FadA, a virulence factor present on the cell surface of *Fusobacterium,* to the E-cadherin of colonocytes. Additionally, FadA would alter the activity of E-cadherin through the activation of the β-catenin signalling pathway [49]. Once activated, β-catenin acts as a transcriptional coactivator, stimulating the expression of oncogenes, genes that encode growth factors, pro-inflammatory cytokines and genes that regulate the Wnt signalling pathway [1] (Fig. 1).

Studies have observed that individuals with CRC who presented increased *Fusobacterium* population in their tumour tissues also exhibited greater expression of inflammatory genes and proteins, such as cyclooxygenase 2 (COX-2),

Table 2. Main results of studies that compared the intestinal microbiota of the tumour and the matched adjacent tissue of the same individual

Subjects	Sample	Methodology	Main results: Tumour *vs* Adjacent tissue		References
			Greater abundance	**Lower abundance**	
97 Chinese patients with adenocarcinoma	Tumour and non-tumour adjacent tissues (10 cm beyond cancer margins)	qPCR	Genera *Fusobacterium* Species *Enterococcus faecalis and* enterotoxigenic *Bacteroides fragilis*	–	[12]
22 Americans patients with carcinoma	Tumour and non-tumour adjacent tissues (5 cm from the tumour)	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V2	Family *Coriobacteriaceae* Genus *Eikenella*	–	[13]
23 Spanish patients with carcinoma	Tumour and non-tumour adjacent tissues (5 cm from the tumour)	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V2	Genera *Bulleida*, *Butyricimonas*, *Campylobacter, Fusobacterium*, *Gemella*, *Parvimonas* and *Streptococcus*	Genera *Blautia* and *Lachnospira*	[13]
31 Chinese patients with carcinoma	Tumour and non-tumour adjacent tissues (5 cm from the tumour)	Pyrosequencing of the 16S rRNA amplicons covering variable regions V3	Genera *Bacteroides*, *Fusobacterium*, *Lactococcus*, *Prevotella* and *Streptococcus*	Genus *Pseudomonas*	[10]
10 Japanese patients with serrated carcinoma	Tumour and non-tumour tissues	qPCR	Species *Fusobacterium nucleatum*	–	[14]
55 British patients with adenocarcinoma	Tumour and non-tumour adjacent tissues	qPCR	Genus *Fusobacterium*	–	[15]
122 European patients with CRC	Tumour and non-tumour tissues	qPCR	Species *Fusobacterium nucleatum*	–	[16]
89 Americans with primary carcinoma	Tumour and non-tumour adjacent tissues	qPCR	Species *Fusobacterium nucleatum* and *Fusobacterium pan-fusobacterium*	–	[17]
8 Chinese patients with carcinoma	Tumour and non-tumour adjacent tissues	qPCR of the 16S rRNA amplicons covering variable regions V1 to V2	Genus *Roseburia*	Genera *Anoxybacillus* and *Microbacterium*	[18]
19 Americans patients with CRC	Tumour and non-tumour adjacent tissues	Pyrosequencing of the 16S rRNA amplicons covering variable regions V3 to V4	Genera *Fusobacterium*	–	[19]
29 Americans patients with adenoma	Adenoma tissue and non-adenoma adjacent tissue	qPCR	Genera *Fusobacterium*	–	[20]
65 Americans patients with CRC	Tumour and non-tumour tissue	Metatranscriptomic	Genera *Campylobacter, Fusobacterium, Leptotrichia* and *Selenomonas*	Families *Ruminococcaceae*; genera *Holdemania, Parabacteroides, Pseudoflavonifractor* and *Ruminococcus*	[21]

nuclear factor kappa B (NF-κB), tumour necrosis factor alpha (TNF-α), interleukin (IL)-12, IL-8 and IL-6, as well as matrix metalloproteinase (MMP) 3 and 9, which contribute to tumour development and the occurrence of metastases [19, 20, 40, 49].

After *Fusobacterium* invades the submucosa, it is able to recruit myeloid-derived suppressor cells through the production of chemotactic compounds, such as N-formyl-methionyl-leucyl-phenylalanine and short-chain fatty acids (SCFAs). These immune cells can suppress the activity of $CD4^+$ T cells through the expression of arginase-1 and inducible nitric oxide synthase [20]. Furthermore, Gur *et al.* [50] observed that *F. nucleatum* inhibits the cytotoxic activity of natural killer cells and tumour-infiltrating lymphocytes by binding its Fap2 protein to the immune inhibitory receptor TIGIT, which would allow the tumour cells to evade the immune response (Fig. 1).

Studies have suggested that *Fusobacterium* exerts its carcinogenic effect in the colonocytes after the adenomatous polyposis coli (*APC*) gene losses its tumour suppressing activity [20, 49], a limiting step for the initiation of carcinogenesis [1]. Thus, an increase in the population of *Fusobacterium* would occur in the early stages of tumour development (adenoma) and would continuously increase as the tissue becomes more

Table 3. Case–control studies that evaluated the intestinal microbiota from faecal samples from individuals with CRC

Subjects	Sample	Methodology	Main results: Caso(s) *vs* Control		References
			Greater abundance	Lower abundance	
11 Moroccan CRC patients and 12 healthy controls	Faecal samples	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V2	Species *Collinsella aerofaciens*, *Oxalobacter formigenes*, *Akkermansia muniphila* and *Bacteroides fragilis*	Species *Prevotella copri*, *Prevotella stercorea*, and *Faecalibacterium prausnitzii*	[22]
39 Swedes with CRC and 66 healthy controls	Faecal samples were collected prior to the colonoscopy	qPCR	Species *Fusobacterium nucleatum* and colibactin-producing *Escherichia coli*	–	[23]
233 Americans with adenoma (≥1 cm) and 547 controls with no polyps	Faecal samples were collected prior to the colonoscopy	qPCR	Genera *Bilophil*a, *Desulfovibrio*, *Sutterella* and *Mogibacterium*	Genera *Veillonella* and *Haemophilus*	[24]
59 Irish patients with carcinoma and 56 healthy controls	Faecal samples were collected prior to the start of the bowel preparation	qPCR of the 16S rRNA amplicons covering variable regions V3 to V4	–	Genus *Coprococcus* Species *Lachnospiraceae incertae sedis*	[9]
203 Asians patients with adenoma and 236 healthy controls	Faecal samples were collected before or 1 month after the colonoscopy	qPCR	Species *Fusobacterium nucleatum* and *Clostridium hathewayi*	Species *Bacteroides clarus* and *Roseburia intestinalis*	[25]
42 Americans newly diagnosed with adenocarcinoma prior to surgery and treatment, and 89 health controls matched by age and BMI	2-day faecal samples	qPCR of the 16S rRNA amplicons covering variable regions V3 to V4	Genera *Fusobacterium* and *Porphyromonas*	Families *Clostridia* and *Lachnospiraceae*	[26]
41 Austrian patients with carcinoma, 42 with advanced adenoma and 55 healthy controls	Fresh faecal samples	Paired-end metagenomic sequencing	Genera *Bacteroides* and *Fusobacterium* Species *Peptostreptococcus stomatis*	Genus *Ruminococcus* Species *Bifidobactium animalis* and *Streptococcus thermophilus*	[27]
41 Austrian patients with carcinoma and 42 with advanced adenoma	Fresh faecal samples	Paired-end metagenomic sequencing	Genera *Bacteroides* and *Parabacteroides* Species *Alistipes putredinis*, *Bilophila wadsworthia, Escherichia coli, Gemella morbillorum, Lachnospiraceae bacterium* and *Pavimonas micra*	–	[27]
42 Austrian patients with advanced adenoma and 55 healthy controls	Fresh faecal samples	Paired-end metagenomic sequencing	Genera *Bacteroides* and *Fusobacterium* Species *Peptostreptococcus stomatis*	Genus *Ruminococcus* Species *Bifidobactium animalis* and *Streptococcus thermophilus*	[27]
17 Brazilians patients with carcinoma and 10 health controls	Faecal samples were collected 2 days before the colonoscopy	qPCR	Species *Fusobacterium nucleatum* and *Clostridium difficile*	–	[28]
20 Chinese patients with precancerous adenoma and 24 health controls	Faecal samples were collected 2 weeks before the colonoscopy	qPCR of the 16S rRNA amplicons covering variable regions V3 to V4	Families *Enterobacteriaceae*, *Gammaproteobacteria*, *Halomonadaceae*, *Legionellaceae* and *Pseudomonadaceae* Genera *Escherichia, Pantoea, Salmonella, Serratia, Shigella, Morganella* and *Trabulsiella*	–	[29]

Continued

Table 3. Continued

Subjects	Sample	Methodology	Main results: Caso(s) *vs* Control		References
			Greater abundance	Lower abundance	
7 Spanish patients with adenocarcinoma and 10 health controls	Faecal samples were collected 1 week before the colonoscopy	Pyrosequencing of the 16S rRNA and qPCR	Genera *Prevotella* and *Methanobrevibacterium* Species *Blautia coccoides* and *Fusobacterium nucleatum*	Genus *Bifidobacterium*	[30]
11 Spanish patients with tubular adenoma and 10 health controls	Faecal samples were collected 1 week before the colonoscopy	Pyrosequencing of the 16S rRNA and qPCR	Genus *Prevotella* Species *Blautia coccoides*	–	[30]
7 Irish patients with adenomas, 24 patients with adenomas and 25 healthy controls	Fresh faecal samples	qPCR	Species *Fusobacterium nucleatum*	–	[16]
30 North American patients with adenoma and 30 health controls	Faecal samples were collected after the usual dietary and medication restrictions had been followed for 24 h	qPCR of the 16S rRNA amplicons covering variable region V4	Families *Clostridium*, *Porphyromonadaceae* and *Ruminococcaceae* Genus *Pseudomonas*	Family *Lachnospiraceae* Genera *Bacteroides* and *Clostridium*	[31]
30 North American patients with carcinoma and 30 health controls	Faecal samples were collected after the usual dietary and medication restrictions had been followed for 24 h	qPCR of the 16S rRNA amplicons covering variable region V4	Families *Enterobacteriaceae* and *Lachnospiraceae* Genera *Fusobacterium* and *Porphyromonas*	Family *Clostridiales* Genus *Bacteroides*	[31]
30 North American patients with carcinoma and 30 patients with adenoma	Faecal samples were collected after the usual dietary and medication restrictions had been followed for 24 h	qPCR of the 16S rRNA amplicons covering variable region V4	Genera *Bacteroides, Fusobacterium, Phascolarctobacterium* and *Porphyromonas*	Family *Lachnospiraceae* Genera *Blautia, Clostridium* and *Ruminococcus*	[31]
47 Americans newly diagnosed with adenocarcinoma and 94 health controls matched by sex and BMI	2-day faecal samples	Pyrosequencing of the 16S rRNA amplicons covering variable regions V3 to V4 and qPCR	Genera *Atopobium, Fusobacterium* and *Porphyromonas*	Class *Clostridia* Family *Lachnospiraceae* Genus *Coprococcus*	[32]
6 African-American patients with colon polyp and 6 healthy controls matched by demographic parameters	Fresh faecal samples	Phylogenetic microarray (human intestinal tract chip analysis) and pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V3	Genus *Bacteroides*	–	[33]
47 Chinese patients with advanced adenoma and 47 health controls matched by sex and age	Fresh faecal samples	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V3 and qPCR	Genera *Bacteroidetes, Enterococcus* and *Streptococcus*	Genera *Clostridium, Eubacterium* and *Roseburia*	[34]
27 Americans patients with carcinoma, 29 patients with adenoma and 30 health controls	Faecal samples were collected prior to the start of the bowel preparation	qPCR	Genus *Fusobacterium*	–	[20]

Continued

Table 3. Continued

Subjects	Sample	Methodology	Main results: Caso(s) *vs* Control		References
			Greater abundance	Lower abundance	
10 recently diagnosed American colon cancer patients prior to surgery for colonic resection and 11health controls	Faecal samples were collected prior to surgery for colonic resection	Pyrosequencing of the 16S rRNA amplicons covering variable region V4	Genera *Acidaminobacter* and *Phascolarcbacterium* Species *Akkermansia muciniphila* and *Citrobacter farmer*	Species *Bacteroides capillosus, B. finegoldii, B. intestinallis, Dialister invisus, D. pneumosintes, Dorea formicigenerans, Lachnobacterium bovis, Lachnospira pectinoschiza, Megamonas hypermegale, Prevotella copri, Pseudobutyrivibrio ruminis, Ruminococcus albus* and *R. obeum*	[35]
19 Chinese patients with CRC and 20 health controls matched by age, sex and BMI	Fresh faecal samples	Pyrosequencing of the 16S rRNA amplicons covering variable region V3 and qPCR	Genera *Alistipes, Bacteroides, Blautia, Dorea, Escherichia/Shigella, Fastidiosipila, Odoribacter, Oscillibacter, Phascolarctobacterium, Ruminococcus* and *Subdoligranulum*	Genera *Faecalibacterium* and *Roseburia*	[36]

BMI: body mass index; CRC: colorectal cancer; DGGE: denaturing gradient gel electrophoresis; qPCR: quantitative polymerase chain reaction; RISA: ribosomal intergetic spacer analysis.

dysplastic, until it reaches the carcinoma stage [16, 20, 51]. In this light, *Fusobacterium* could be considered to be a passenger bacterium that may perpetuate tumorigenesis through inappropriate stimulation of the colon epithelial cells and the local immune system.

The tumour tissue provides a conducive environment for the proliferation of *Fusobacterium*. This is because the tumour tissue and *Fusobacterium* do not compete for the same energy substrate, with the latter being an asaccharolytic bacterium, which preferentially uses amino acids and peptides instead of carbohydrate, as the tumour tissues do [20].

Streptococcus gallolyticus subsp. *gallolyticus* (Sgg), formerly *Streptococcus bovis* biotype I, is a Gram-positive opportunistic pathogen that is detected in the human intestinal microbiota in low abundance (2.5–15%) [52]. Over the last 50 years, endocarditis caused by Sgg has been considered to be a strong indicator of colonic diseases [53, 54]. However, there is still controversy about whether Sgg endocarditis is a consequence of CRC or an aetiological factor for this type of cancer.

The presence of Sgg in blood has become an indication for colonoscopy. Corredoira-Sánchez *et al.* [55] observed that 70% of patients with Sgg blood infection who underwent colonoscopy were diagnosed with CRC; in contrast, only 32% of patients with Sgg infection who had symptoms or a family history of CRC were diagnosed.

The mechanism by which Sgg triggers the onset or progression of CRC development is not fully understood. However, it is known to have low adhesion (<15%) to healthy colonocytes [56]. Thus, the colonization capacity of Sgg in the intestine of healthy individuals is low. Interestingly, Sgg is able to adhere to collagen-rich surfaces using its collagen-binding proteins and pilus [57, 58]. Given that pre-neoplastic and/or neoplastic lesions present a higher quantity of collagen than healthy colonic tissue [56, 59], Sgg has a competitive advantage in the colonization of these regions [60, 61]. It is probable that throughout the tumour development phase this bacterium can express other types of pilus that are suitable for the colonization of new microenvironments [62].

Sgg produces gallocin (*gallo_2020*, *gallo_2021* and *gallo_2203*), a type of bacteriocin that acts on target bacterium, increasing membrane permeability. Thus, the colonization of pre-neoplastic and/or neoplastic lesions by Sgg is facilitated compared to Gram-positive bacteria [61].

In addition, the end products of glucose metabolism produced by tumour cells, such as fructose-6-phosphate and glucose-3-phosphate, can serve as energy substrates for Sgg, and so this microenvironment contributes to the colonization and proliferation of these bacteria [63].

After adhering to tumours, Sgg can translocate across the epithelial monolayer via a paracellular mechanism [56]. On the lamina propria, Sgg is relatively invisible to the epithelial immune system, since its surface is covered by a polysaccharide coating, which causes delayed recruitment of tissue macrophages [52, 57]. After being captured by macrophages, Sgg stimulates the local production of inflammatory mediators, such as COX-2, pro-inflammatory cytokines (IL-8, IL-1β, TNF-α e IL-6) and free radicals [56].

Table 4. Case–control studies that evaluated the intestinal microbiota from tissue samples of individuals with CRC

Subjects	Sample	Methodology	Main Results: Case(s) *vs* Control		References
			Greater abundance	Lower abundance	
15 Swedish patients with stage I to III CRC and 21 healthy controls	Tissue sample was collected during colonoscopy	qPCR of the 16S rRNA amplicons covering variable regions V	Genera *Fusobacterium*, *Methanobrevibacter*, *Clostridium*, *Dialister*, *Peptostreptococcus*, *Selenomonas*	Genus *Streptococcus*	[37]
97 African-Americans with adenocarcinoma and 100 healthy controls	Case: undiseased tissue located at 10 cm away from the tumour location Control: normal tissue	Pyrosequencing of the 16S rRNA amplicons covering variable region V4 and qPCR	Genus *Pyramidobacter* Species *Bilophila wadsworthia*	–	[38]
52 Chinese patients with invasive adenocarcinomas and 61 healthy controls	–	Pyrosequencing of the 16S rRNA amplicons	Genera *Campylobacter*, *Dialister*, *Fusobacterium*, *Leptotrichia*, *Mogibacterium*, *Parvimonas*, *Peptostreptococcus*, *Lactobacillus* and *Prevotella*	Genera *Acidomonas*, *Escherichia*, *Pseudomonas*, *Sphingomonas*, *Blautia* and *Faecalibacterium*	[39]
59 Irish patients with carcinoma and 56 healthy controls	Case: undiseased tissue located 10 to 30 cm from the distal or proximal tumour Control: two biopsies per individual, one from the descending colon and one from the ascending colon	qPCR of the 16S rRNA amplicons covering variable regions V3 to V4	Genera *Bacteroides*, *Fusobacterium*, *Oscillibacter*, *Parvimonas*, *Peptostreptococcus*, *Porphyromonas*, *Roseburia*, and *Ruminococcus*	–	[9]
28 Chinese patients who died within 3 years after surgery for CRC-related causes and 92 patients who lived more than 3 years without any sign of recurrence or metastasis (controls)	Tumour samples (stages I to IV)	qPCR of the 16S rRNA amplicons covering variable region V4	Genus *Fusobacterium* Species *Bacteroides fragilis*	Genera *Faecalibacterium*, *Methylobacterium*, *Mycoplasma*, *Shewanella* and *Sphingomonas* Species *Faecalibacterium prausnitzii* and *Methylobacterium suomiense*	[40]
97 Chinese patients with adenocarcinoma and 48 health controls matched by sex and age	Case: adjacent non-tumour tissues (10 cm beyond cancer margins) Control: normal tissue	qPCR	Genus *Fusobacterium* Species *Enterococcus faecalis*	–	[12]
31 Chinese patients with carcinoma and 30 health controls	Case: 15 proximal colon cancer tissues and 16 distal colon cancer tissues Control: 15 proximal colon tissues and 15 distal colon tissues	Pyrosequencing of the 16S rRNA amplicons covering variable region V3	Genera *Escherichia–Shigella*, *Fusobacterium*, *Lactococcus* and *Peptostreptococcusten*	Genera *Acidovorax*, *Acinetobacter*, *Brevundimonas*, *Buttiauxella*, *Caulobacter*, *Epilithonimonas*, *Flavobacterium*, *Janthinobacterium*, *Pedobacter*, *Propionibacterium Pseudomonas*, *Psychrobacter*, *Rahnella*, *Stenotrophomonas*, *Sphingobacterium* and *Sphingomonas*	[10]
343 Japanese patients with sessile serrated adenoma and 122 non-serrated adenoma (controls)	Case: adenoma tissue Control: normal tissue	qPCR	Similar amounts of *Fusobacterium nucleatum* species among the different histopathological groups		[14]

Continued

Table 4. Continued

Subjects	Sample	Methodology	Main Results: Case(s) *vs* Control		References
			Greater abundance	Lower abundance	
7 Spanish patients with adenocarcinoma and 10 health controls	Normal mucosa from the rectum	Pyrosequencing of the 16S rRNA and qPCR	Family *Enterobacteriaceae* Genera *Bacteroides, Blautia* and *Prevotella* Species *Akkermansia muciniphila, Blautia coccoides* and *Fusobacterium nucleatum*	–	[30]
11 Spanish patients with tubular adenoma and 10 health controls	Normal mucosa from the rectum	Pyrosequencing of the 16S rRNA and qPCR	Genera *Escherichia–Shigella, Bacteroides, Blautia* and *Prevotella* Species *Blautia coccoides*	Genera *Bacillus* and *Staphylococcus*	[30]
7 Spanish patients with adenocarcinoma and 11 patients with tubular adenoma	Normal mucosa from the rectum	Pyrosequencing of the 16S rRNA and qPCR	Families *Enterobacteriaceae* and *Methanobacteriales* Species *Fusobacterium nucleatum*	–	[30]
10 Chinese patients with adenoma and 10 health controls	Case: adenoma tissue Control: normal tissue	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V2	Family *Streptococcaceae* Genera *Parascardovia, Porphyromonas, Satphylococcus, Streptococcus, Streptophyla* and *Veillonella*	Genera *Anoxybacillus, Megamonas, Methylobacterium* and *Microbacterium*	[41]
8 Chinese patients with carcinoma and 10 health controls	Case: tumour tissue Control: normal tissue	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V2	Families *Enterobacteriaceae*, *Pseudomonadaceae* and *Neisseriaceae* Genera *Chryseobacterium*, *Enterobacter*, *Parascardovia, Planomicrobium* and *Streptococcus*	Genera *Anoxybacillus, Megamonas, Methylobacterium* and *Microbacterium*	[41]
30 Czech patients with carcinoma and 20 healthy controls	Mucosal biopsies were taken from the caecum, transverse colon and the rectum in all the cases and controls	Culture in specific medium	Phylogroup *Escherichia coli* B2	–	[42]
6 Czech patients with advanced colorectal adenoma and 24 with non-advanced adenoma (control)	Mucosal biopsies were taken from the caecum, transverse colon and the rectum in all the cases and controls	Culture in specific medium	Phylogroup *Escherichia coli* B2	–	[42]
15 Americans patients with adenoma and 15 health controls	Normal rectal mucosa, approximately 10 to 12 cm from the anal verge	qPCR	Genera *Eubacteria* and *Bifidobacterium*	–	[43]
50 French patients with resectable CRC and 33 uncomplicated diverticulosis non-cancer controls	Case: non-necrotic fragment from the peripheral areas of the tumour Control: normal mucosa (absence of diverticules)	PCR	Species *Escherichia coli*	–	[44]
38 France patients with CRC and 31 patients with complicated diverticulosis (control)	Case: mucosal biopsies from the proximal or distal colon Control: mucosal biopsies from the sigmoid colon	Culture in specific medium, ERIC-PCR and RAPD-PCR	Phylogroup *Escherichia coli* B2	–	[42]

Continued

Table 4. Continued

Subjects	Sample	Methodology	Main Results: Case(s) *vs* Control		References
			Greater abundance	Lower abundance	
48 Americans patients with adenoma and 67 health controls matched by sex and age	Normal mucosa from the rectum	Pyrosequencing of the 16S rRNA amplicons covering variable regions V1 to V3 and qPCR	Genera *Fusobacterium*	–	[19]

BMI: body mass index; CRC: colorectal cancer; ERIC-PCR: enterobacterial repetitive intergenic consensus sequence-PCR using primer; qPCR: quantitative polymerase chain reaction; FISH: clone sequence analysis and florescence *in situ* hybridization; RAPD-PCR: random amplified polymorphic DNA-PCR; T-RFLP: terminal restriction fragment length polymorphism.

The inflammatory response triggered by Sgg could initiate the development of CRC [64]. IL-8 stands out among the inflammatory cytokines expressed in individuals who have CRC and an abundant Sgg population in their intestinal microbiota [65, 66]. This cytokine stimulates angiogenesis and vasodilatation and enhances capillary permeability, contributing to tumour development and acting as a possible pathway for the entry of Sgg into the bloodstream [64].

Sgg can stimulate the proliferation of colon cancer cells by increasing the expression of β-catenin and its oncogenic downstream targets, such as c-Myc, and the proliferation of cell nuclear antigen (PAN) [60, 67]. However, the proliferation-promoting effect of Sgg is dependent on the phase of bacterial growth (the stationary phase stimulates cell proliferation, while the exponential phase does not) and direct contact between live bacteria and tumour cells [67].

The classification of Sgg as driver or passenger is controversial [60, 61, 64, 67]. As discussed, for this bacterium to colonize the colon and further stimulate tumour development, there should be at least a mutation in the *APC* gene [61, 67]. Therefore, Sgg cannot be considered to be a major cause of CRC, although its presence in a pre-neoplastic microenvironment accelerates the development of CRC.

Bacteria commonly involved in the inflammatory diseases of the gastrointestinal tract have been found in larger

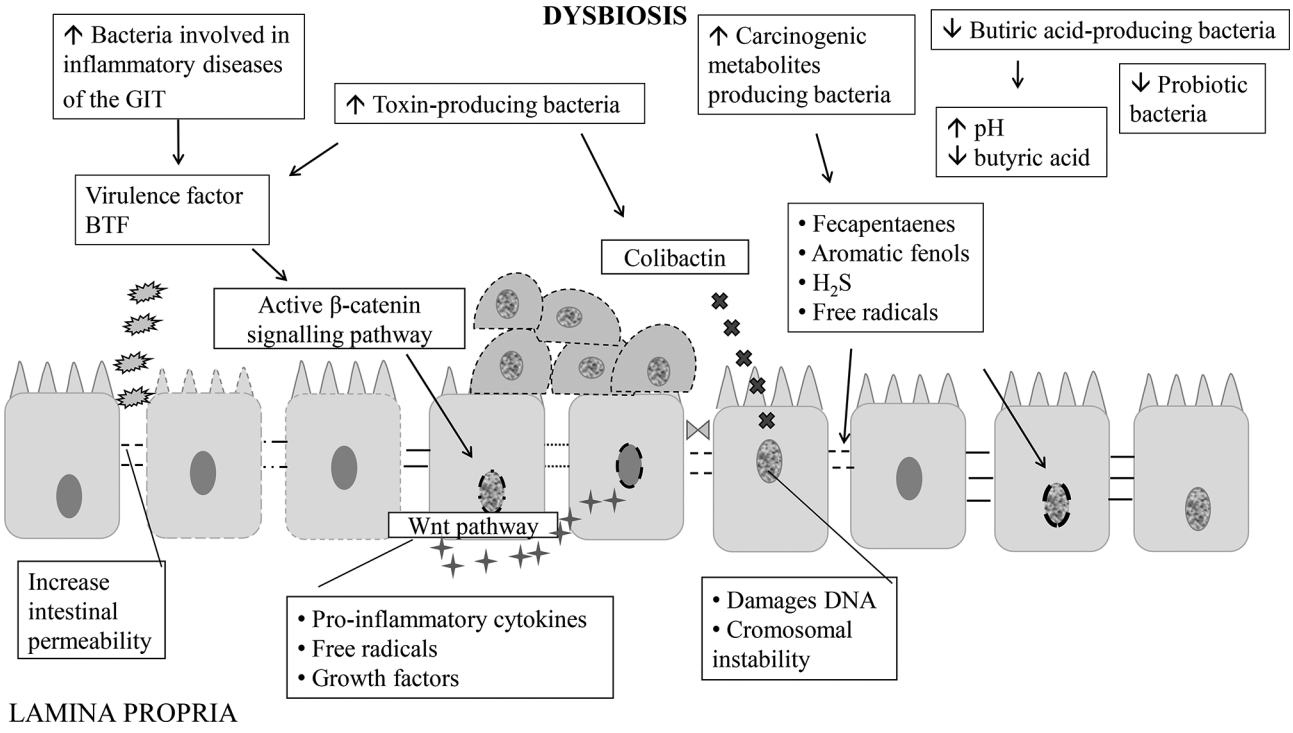

Fig. 1. A colonic dysbiosis may occur prior to or during tumour development; an increase in bacterial populations related to the intestinal inflammatory diseases, producing toxins and carcinogenic metabolites, has been observed, while a reduction in the production of short-chain butyric fatty acids and probiotics may also be observed.

amounts in the intestinal microbiota of individuals with CRC (Tables 1–4). Among these bacteria are: *Atopobium*, which is related to Crohn's disease [68]; *Porphyromonas* and *Prevotella*, which are commonly associated with periodontal diseases [69, 70]; *Citrobacter*, which is found in larger amounts in the intestinal microbiota of individuals with inflammatory bowel disease [71, 72]; and *Shigella*, a well-known aetiological agent of diarrhoea [10].

In general, these groups of bacteria appear to contribute to CRC development by triggering a chronic inflammatory response that stimulates the uncontrolled proliferation of existing pre-neoplastic cells, thus acting as passenger bacteria.

Toxin-producing bacteria

The presence of toxin-producing bacteria in the intestinal microbiota can be related to the increased risk of CRC because the toxins synthesized by these bacteria can bind to specific receptors on the surface of colonocytes, altering intracellular signalling and resulting in disordered cell proliferation and differentiation [44, 73].

In this way, enterotoxigenic *Bacteroides fragilis* (ETBF) have been found in larger amounts in the colon of individuals with CRC (Tables 1–4). Experiments with Min mice (heterozygous for the *APC* gene) showed that ETBF induces the formation of colonic tumours, mainly in the distal regions of the colon, unlike non-toxin-producing *Bacteroides fragilis* (NTBF). In addition, these tumours were typically laden with inflammatory infiltrates (granulocytes and mononuclear cells), which were not observed in the tumours of NTBF-colonized mice [74].

ETBF is distinct from other *Bacteroides* species due to the secretion of a zinc-dependent metalloprotease (20 kDa) toxin, called *B. fragilis* toxin (BFT). This toxin can bind to putative colonic epithelial cells receptors, triggering the cleavage of E-cadherin. This process increases intestinal permeability [75] and releases E-cadherin-associated β-catenin. As a result, the Wnt pathway is activated, which stimulates the expression of oncogenes [76]. BTF also triggers the activation of the NF-κB pathway, resulting in the secretion of proinflammatory cytokines, such as IL-6, in the intestinal epithelial cell [74].

Increased intestinal permeability contributes to the selective activation of Stat3 by ETBF, initially in the immune cell followed by the colonic epithelial cells [77, 78]. Stat3 belongs to a family of transcription factors that play a crucial role in the regulation of immune responses. In this way, Stat3 induces a strong infiltration of the lamina propria by IL-17-producing $CD4^+$ T cells (T helper 17) and stimulates IL-17 transcription. In addition, IL-12 production is inhibited, which prevents the differentiation of naive T cells into Th1 cells. Further, IL-23 production is stimulated, which promotes the expansion and stabilization of Th17 cells [74].

$FoxP3^+$ Tregs are known as suppressors of inflammatory response and central keepers of peripheral tolerance. However, Tregs are heterogeneous in function and include not only suppressive T cells, but also nonsuppressive ones that secrete proinflammatory cytokines, such as IL-17 [79]. Non-suppressive T cells originate from Treg lineages with the *RORC* gene, a key transcription factor for Th17 cell lineage [80]. Thus, these Treg cells secrete proinflammatory cytokines, such as IL-17, and, depending on the environmental stimuli, such as cytokines, intestinal microbiota composition and their metabolites/toxins, they can differentiate into $RORC^+FoxP3^+CD4^+$ T cells, which share the same phenotype with both Th17 and Tregs, contributing to ETBF-triggered inflammatory response [81].

Further, the increased intestinal permeability triggered by BFT may attract the Treg cells to that place. As the survival of Treg is dependent on IL-2 and they are unable to produce it, these cells consume the available IL-2 in the environment. In this way, the inhibitory stimulus of IL-2 on the differentiation of T cells in Th17 is reduced, which contributes to the local expansion of the Th17 cell population. However, such a contribution would only occur in the early stages of tumour development triggered by ETBF [79].

While investigating the importance of IL-17 in the initial tumour development promoted by ETBF, Wu *et al.* [74] found that Min mice colonized with ETBF and treated with IL-17A-blocking antibodies exhibited a reduced number of colonic tumours compared to the untreated control. Furthermore, Housseau *et al.* [78] observed that 8 weeks after ETBF colonization, Min-$CD4^{Stat3-/-}$ mice exhibited a lower number of colonic tumours compared to parental Min mice.

A high local concentration of IL-17 stimulates the expression of chemokines (CXCL1, CXCL2 and CXCL5), which attracts myeloid cells to the tumour environment [82]. In this environment, BFT stimulates the polarization of immature myeloid cells to monocytic myeloid-derived suppressor cells. These cells inhibit the antitumour immune response, especially that of $CD8^+$ T cells, which would contribute to tumour development. Further, monocytic myeloid-derived suppressor cells produce IL-23, which attracts more Th17 cells to the tumour site [83].

In addition to the effects of IL-17 on immune cells, this cytokine can stimulate the expression of anti-apoptotic genes in tumour cells, such as Bcl-x_L e Bcl-2, which increases the survival of these cells [84].

BFT can stimulate tumour development by increasing the local production of reactive oxygen species (ROS). This toxin stimulates the expression and activity of the enzyme spermine oxidase that converts polyamine spermine into spermidine. In this process, hydrogen peroxide (H_2O_2), is produced, which causes inflammation and DNA damage, contributing to tumour development [85].

The classification of ETBF as a driver or passenger is controversial because this bacterium can be found in both the early and final phases of tumour development (Tables 1–4). In addition, Wu *et al.* [74] observed that the activation of Stat3 by ETBF occurs regardless of mutation in the *APC* gene, which suggests that this bacterium can act as a driver.

Some members of the family *Enterobacteriaceae*, such as enterotoxigenic *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Citrobacter koseri*, have also been observed in larger quantities in the intestinal microbiota of individuals with CRC (Tables 1–4). They contain the biosynthetic gene island (*clbA* to *clbS*) called polyketide synthase islands (pks), which is responsible for colibactin production. Colibactin is a natural genotoxin whose structure is not yet known due to its low stability, a property that limits studies on it [86].

The mechanisms through which colibactin interacts with the colonocyte and damages its DNA are not yet fully understood [87]. However, it has been observed that for this toxin to damage the DNA, the eukaryotic cell must interact directly with the toxin-producing bacteria, and the bacteria need to be alive. In addition, treatment of the eukaryotic cell with the supernatant of the bacterial culture does not damage the DNA molecule [88].

Intestinal cell infection (*in vitro* and *in vivo*) with *pks*$^{+}$ *E. coli* induces DNA double-strand breaks (DSBs) and interstrand cross-links (ICLs) (Fig. 1) [88, 89]. In response to this damage, the DNA damage signalling cascade is activated.

DNA mismatch repair (MMR) proteins maintain genomic stability by correcting DNA base pair mismatches. Defective DNA MMR confers a mutator phenotype, leading to the accumulation of thousands of somatic mutations in the DNA microsatellite sequences (microsatellite instability) [90]. To repair DNA ICLs, the Fanconi pathway is activated. In this sense, Fanconi anaemia protein D2 (FANCD2) is activated by monoubiquitinylation and is recruited to stalled forks, where it promotes ICL removal [88]. However, not all damaged DNA is repaired, in particular because the toxin itself is able to negatively regulate the expression of proteins involved in the repair pathway [90]. Thus, with continuous cell cycles, the unrepaired cells propagate chromosome aberrations [89].

Colibactin can induce an irreversible cell cycle arrest, which leads to premature cellular senescence [91]. For this purpose, colibactin upregulates microRNA-20a-5p, which silences the transcription of SENP1, a key enzymatic regulator of p53 [92]. This senescence would trigger cellular reprogramming, which would increase the cellular production of ROS and pro-inflammatory mediators (IL-6 and MMP) [91], agents that can cause damage to neighbouring cells, contributing to tumour development.

The increase of he family *Enterobactericeae* in the microbiota of individuals with CRC could be related to the availability of energy substrate in the tumour microenvironment, in the form of products derived from the inflammatory response, e.g. nitrate [93]. Further, colonization may depend on the interaction between the bacterium and the host receptor known as carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), which is produced abundantly in tumour cells [87].

Thus, the family *Enterobactericeae* could be classified as drivers, since they can cause initial damage to the DNA molecule, which would trigger tumour development. However, more studies are needed, since some researchers question the relationship between enterotoxigenic *E. coli* and the development of CRC [87].

Carcinogenic metabolite-producing bacteria

The great microbial diversity present in the colon allows the daily production of infinite metabolites. Although some metabolites are essential for host health, some bacteria can produce potentially carcinogenic compounds from the enzymatic metabolization of inactive compounds (Fig. 1), such as dietary components, pharmaceuticals and compounds produced by the host itself [73].

It has been observed that individuals with CRC have larger amounts of carcinogenic metabolite-producing bacteria in their intestinal microbiota (Tables 1–4). These bacteria can be classified as drivers or passengers, depending on the stage of tumour development, since the metabolites produced can contribute to the initiation and development of CRC.

Glucuronidation is a major detoxification process that mainly occurs in the liver and converts xenobiotic and endogenous substances into more hydrophilic metabolites. After conversion, these substances are sent to the intestinal lumen, along with the bile, to be eliminated from the body through faeces. However, bacteria that present β-glucuronidase and β-glycosidase enzymes can break the bond between glucuronic acid and the xenobiotic substance, allowing the substance to function freely in the body, which increases the risk of developing CRC [94]. To exemplify the importance of these enzymes in the risk of CRC, the 1,2-dimethylhydrazine compound that is widely used in the chemical induction of CRC in animal models only exerts its carcinogenic effect after being metabolized by β-glucuronidase. This enzyme will deconjugate glucuronic acid from the 1,2-dimethylhydrazine molecule, allowing the latter to exert its carcinogenic activity [95].

In the intestinal microbiota, the enzyme β-glycosidase appears to be more common than β-glucuronidase [96]. These enzymes have been identified in some bacteria that comprise the intestinal microbiota, such as *B. fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Bifidobacterium dentium, Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium longum*, *Blautia hansenii, Butyrivibrio fibrisolvens, Clostridium perfringens, E. coli, Eubacterium eligens, Eubacterium rectale, Faecalicabterium prausnitzii, Lactobacillus gasseri, Lactobacillus jensenii, Lactobacillus rhamnosus, Roseburia intestinalis, Roseburia faecis, Ruminococcus gnavus*, etc. [94, 96].

It should be emphasized that the intestinal activity of these enzymes is not always harmful; their action can be essential for the activation of beneficial compounds such as polyphenols [97]. Thus, the presence of these enzymes in the colon should be evaluated in conjunction with the lifestyle of the

individual, such as dietary patterns and medication use [96]. In addition, there are different isoforms of these enzymes that must be investigated with regard to their potential to produce carcinogenic substances [98].

Nitrocompounds (nitrate, dinitrotoluene, nitrobenzene, *N*-nitrosodimethylamine, *N*-nitrosamines, *N*-nitrosamides, *N*-nitrosoguanidines, *N*-nitrosoureas and nitropyrene) can enter the body through food, medication, and even through the air we breathe. In the body, these compounds can be metabolized by bacterial enzymes, such as nitroreductases, to N-nitroso compounds (heterocyclic and aromatic nitro compounds), many of which are highly carcinogenic, as they can act as DNA-alkylating agents [99]. Nitroreductase enzymes can be found in some species of the genera *Acidovorax*, *Citrobacter*, *Desulfovibrio*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Neisseria*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella* and *Vibrio* [100].

Azo dyes (methyl red; sudan I, II, III and IV; sunset yellow; allura red; para red; orange G; etc.) are a group of compounds containing one or more R1–N=N–R2 bonds. These types of dyes represent the majority of dyes used in society today [101]. Azo dyes can be biotransformed into aromatic amines by azoreductases, enzymes that catalyze the reductive cleavage of azo groups [102]. This enzyme can be found in a large number of bacterial species, such as *B. fragilis*, *B. thetaiotaomicron*, *B.* adolescentis, *Bifidobacterium infantis*, *Clostridium nexile*, *Clostridium perfringens*, *Clostridium clostridiiforme*, *Clostridium paraputrificum*, *Clostridium sporogenes*, *Clostridium ramosum*, *E. aerogenes*, *Enterococus faecalis*, *E. coli*, *F. prausnitzii*, *Klebsiella aerogenes*, *Lactobacillus catenaforme*, *Pseudomonas aeruginosa*, *Ruminocossus brommi*, *Peptostreptococcus productus*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Veillonella parvula* [99].

Primary bile acids, which are naturally produced in the liver, can be converted into secondary bile acids, such as deoxycholic and lithocholic acid, by the enzyme 7α-dehydroxylase, which is found in the intestine. When present in large quantities, secondary bile acids may increase the risk of developing CRC, as they increase local production of free radicals;,stimulate PGE2 synthesis, activate the *β*-catenin/Wnt signalling pathway and alter the intestinal barrier. Furthermore, secondary bile acids can prevent the repair of damaged DNA and allow cancer cells to become resistant to apoptosis [103].

Aymerica *et al.* [61] observed that the presence of the secondary bile acids increases the activity of the bacteriocin, gallocin, produced by Sgg. For gallocin to exert its antimicrobial activity, its peptide structure must have a double α-helice configuration, which only occurs when the peptides are in a hypodrophobic environment. Thus, the presence of secondary bile acids contributes to the colonization of the intestine by Sgg.

The 7α-dehydroxylase enzyme appears to be restricted to a limited number of anaerobic bacteria, such as *E.coli, B. fragilis* and *B. thetaiotaomicron*, some *Ruminococcus* and *Clostridium* spp. (*C. absonum*, *C. bifermentans*, *C. hylemonae, C. limosum* and *C. scindens*) [104].

Sulfate-reducing bacteria, such as *Desulfovibrio*, utilize sulfate as an energetic substrate, and during the metabolic process, sulfide is enzymatically converted to hydrogen sulfate [105]. This metabolite has many functions related to the increased risk of developing CRC; it produces free radicals, prevents the activity of cytochrome oxidase, suppresses the use of butyric acid, and inhibits mucus synthesis by the goblet cells and DNA methylation in the colonocytes. Furthermore, secondary bile salts can be metabolized by sulfate-reducing bacteria, increasing hydrogen sulfate production [106].

Additionally, some bacteria that compose the intestinal microbiota, such as *Enterococcus faecalis*, can produce ROS and reactive species of nitrogen as a result of their respiratory process. These free radicals can initiate carcinogenesis by interacting directly or indirectly with the DNA molecule [107].

Given the potential carcinogenic activity of these metabolites, the enumeration and identification of micro-organisms in the intestinal microbiota may not be sufficient to predict the risk of CRC. Thus, the use of metabolomic tools to evaluate potentially carcinogenic metabolites can provide more reliable results. Further, dietary composition should be evaluated, since it often provides substrates for these enzymes. Thus, greater consumption of these substrates can promote the proliferation of carcinogen metabolite-producing bacteria and, consequently, the number of carcinogenic compounds produced.

Butyric acid-producing bacteria

Butyric acid (C4) is a SCFA produced when non-digestible carbohydrate is metabolized by some specific bacteria, such as *Clostridium* cluster XIVa and IV, *Clostridiales bacterium*, *Coprococcus* spp., *E. rectale*, *Faecalibacterium prausnitzii*, *Pseudobutyrivibrio* spp., *R. intestinalis* and *Roseburia inulinivorans* [108].

It has been estimated that a 1 mg l^{-1} reduction of faecal butyric acid concentration is able to increase the risk of developing CRC by up to 84.2% [34]. Further, studies have shown that the population of butyric acid-producing bacteria is lower in the intestinal microbiota of individuals with CRC (Tables 1–4) (Fig. 1).

Butyric acid is related to colonic health because this acid is a major energy source for colonic epithelial cells, which metabolize 70 to 90% of total acid produced [109]. This may also be the reason why butyric acid has different effects on healthy and tumour intestinal cells (Warburg effect). Since tumour cells preferentially metabolize glucose, there is an accumulation of butyric acid in the intracellular medium, which in turn inhibits the activity of the enzyme, histone deacetylase [110]. The inhibition of histone deacetylase leads to increased acetylation of histones, which results in gene silencing during transcription [111].

Among the multiple mechanisms used by butyric acid to inhibit tumour development, its anti-inflammatory action stands out. It has been shown that butyric acid is able to inhibit the expression of up to 31 genes related to the pro-inflammatory immune response in the intestine [112]. Thus, Wei *et al*. [40] observed that tumour tissues of individuals with a low population of *F. prausnitzii* exhibited an increased gene expression of *MMP9*, *TNF* and *CTNNB*, while individuals with a larger population of *F. prausnitzii* exhibited a low expression of NF-κB protein. By inhibiting the action of histone deacetylase, butyric acid suppresses the NF-κB transcription factor that controls the expression of proinflammatory cytokines, chemokines, inducible inflammatory enzymes, adhesion molecules and some growth factors. Thus, through the modulation of this gene and others involved in intestinal immune response, such as PPAR-γ, butyric acid may contribute to the inhibition of tumour development [113].

Butyric acid is also capable of stimulating the expression of antimicrobial peptides, the antioxidant enzyme glutathione-S-transferase, epithelial tight junctions, mucus production and pro-apoptotic factors. In addition, it inhibits tumour angiogenesis, the migration of tumour cells and local production of ROS species, among other things [108–110].

The increase of butyric acid-producing bacteria in the microbiota of individuals with CRC should be evaluated with caution. Butyric acid is also produced by some species of *Bacteroides* and *Fusobacterium* through the metabolism of amino acids and peptides, although phenolic compounds are produced simultaneously, which can damage the DNA of colonocytes [106]. In this context, the dietary intake of the individual should be evaluated, as well as additional metabolites produced by the intestinal microbiota.

Probiotic bacteria and potentially probiotic bacteria

Probiotic or potentially probiotic bacteria are beneficial to their host and have been widely studied for their possible role in the prevention of CRC [114]. The beneficial effect of probiotic bacteria has been proven based on all pre-established criteria, while investigations on potentially probiotic bacteria are still ongoing to prove their probiotic effect [115].

Some mechanisms have been pointed out as being responsible for the anticarcinogenic properties of probiotics. These mechanisms include the following: positive modulation of the intestinal microbiota and consequent alteration of its metabolic activity; binding and degradation of of carcinogenic compounds present in the intestinal lumen; production of anticarcinogenic compounds, such as SCFA and conjugated linoleic acid; immunomodulation; improvement of the intestinal barrier; and induction of apoptosis of tumour cells [114].

Some probiotic and potentially probiotic bacteria belong to the lactic acid bacteria (LAB) group. Feng *et al*. [27] classified LABs as control-enriched metagenomic linkage groups for CRC. This classification may be attributed to some of the aforementioned mechanisms and the lactic acid produced by these bacteria.

Lactic acid reduces the intra-colonic pH, which inhibits the proliferation of potentially pathogenic and putrefactive bacteria, as well as the activity of enzymes responsible for producing potentially carcinogenic compounds [116]. Moreover, the conversion of amino acids to SCFA is inhibited when luminal acidity is high. Lactic acid accelerates cell turnover by promoting daily cell renewal, which allows the elimination of possible neoplastic cells [27].

The population of probiotic and potentially probiotic bacteria can be reduced in the intestinal microbiota of individuals with CRC (Tables 1–4) due to alterations in the tumour microenvironment, which do not favour colonic colonization (Fig. 1).

STUDY LIMITATIONS

Few studies in this review evaluated the diet composition of the included individuals [9, 29, 43, 117, 118] as well as the relationship between diet and the composition of the intestinal microbiota [22, 27, 34, 38]. Given that diet modifies the composition of the intestinal microbiota [119, 120], it should be evaluated whenever possible. In addition, dietary changes after CRC diagnosis should be evaluated with regard to whether they lead to changes in the composition of the intestinal microbiota and their influence on host health.

Another limitation is the difference between the microbiota present in the faeces (transient microbiota) and that found in colon tumour tissue or healthy tissue samples (resident microbiota) [9, 16, 69, 70, 72]. The two microenvironments have different conditions (e.g. energy substrate and adhesion sites), allowing the colonization of different micro-organisms [117]. It is believed that micro-organisms closer or attached to the intestinal mucosa would have a greater influence on carcinogenesis, since their interaction with the colonocytes and immune cells is greater [69, 70]. This suggests that studies conducted with tissue samples (Tables 2 and 4) would have reliable results, and it is therefore recommended that, whenever possible, resident intestinal microbiota should be evaluated.

FUTURE PERSPECTIVES

Different strains of bacteria affect the functioning of the colonic ecosystem in different ways, altering the interaction between micro-organisms and their hosts due to the vast genetic repertoire that codes toxins, enzymes and virulence factors [72]. Thus, future studies should not only investigate the composition of the intestinal microbiota, but also the intestinal microbiome. For instance, the metabolic potential of this microbiota could be explored, as well as its influence on intestinal homeostasis and host health or illness.

Studies have suggested that components of the intestinal microbiota such as viruses, fungi, yeasts, and protozoa can influence the risk of developing CRC [121], and so the relationship between these micro-organisms and CRC risk should be evaluated [122–124].

The real impact of the intestinal microbiota on the outcomes of chemotherapy, radiotherapy and immunotherapy should be investigated, as it could indicate the most appropriate treatment, dosage and duration [125].

CONCLUSIONS

One of the biggest challenges in combating CRC and other types of cancer is their early detection. The establishment of a microbiome profile associated with the risk of developing CRC could help to identify individuals at high risk. Accordingly, the microbiome could serve as a biomarker for risk prediction and diagnosis of CRC.

Based on the microbiota related to the development of colonic carcinogenesis, new strategies to prevent the disease can be studied, since the composition of the intestinal microbiota is modifiable. Further, a personalized approach is possible, according to the microbiota of each individual.

The classification of micro-organisms as 'driver' or 'passenger' could be used as an effective indicator of the phase and development of CRC, given that the composition of the microbiota varies according to the phase of the disease. Moreover, knowledge of the phase of the disease ensures that the best treatment is chosen.

Although studies have shown that the intestinal microbiota of individuals with CRC differs from that of healthy individuals, to date, no single micro-organism or group of specific micro-organisms has been identified as triggering CRC in humans.

These micro-organisms have probably not yet been identified because of the following factors: the type of sample analysed (faeces or tissue); sample collection site (ascending colon, descending colon, sigmoid and rectum); colonoscopy and surgery preparations; sample preparation (freeze-drying or freezing); stage of the disease; inherent characteristics of the participants (age, genetics); geographical differences; participant lifestyle (diet, physical activity, smoking, alcoholism, antimicrobial use, probiotics, prebiotics or symbiotics); sample size, micro-organism identification techniques; and 16 Sr RNA regions studied.

Therefore, more studies should be conducted, in particular multicentre cohort ones employing metagenomic and metabolomic tools, in order to identify the specific microbiome related to CRC and better classify micro-organisms as drivers or passengers. In addition, understanding the synergistic relationship between micro-organisms and hosts will help to clarify the true role of the intestinal microbiota in the development of CRC.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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